

**$\beta$ -CAROTENE AS A POTENTIAL BIOMARKER IN MARTIAN EVAPORITIC ROCKS: EVALUATION OF RAMAN MICROSPECTROMETRIC ANALYSIS.** P. Víték<sup>1</sup>, J. Jehlička<sup>1</sup>, H.G.M. Edwards<sup>2</sup>, K. Osterrothová<sup>1</sup>. <sup>1</sup>Institute of Geochemistry, Mineralogy and Mineral Resources, Charles University in Prague, Albertov 6, 128 43 Prague 2, Czech Republic, vitek2@natur.cuni.cz, <sup>2</sup>Division of Chemical and Forensic Sciences, University of Bradford, Bradford BD7 1DP, United Kingdom.

**Introduction:** Based on the latest results of Martian exploration [1-3], evaporitic rocks on the Martian surface are proposed as potential habitats for microbial (halophilic) life on Mars [4,5]. At least, there is a reasonable possibility that these rocks may sustain molecular remnants (biomarkers) as evidence for the presence of extinct or extant living organisms. It has been shown that Raman spectrometry is a powerful tool for the characterization of various biomarkers which are produced by halophilic or halotrophic microbes as part of their survival strategy [6,7] and in this respect  $\beta$ -carotene is considered a suitable biomarker.

In this work, Raman microspectrometry was tested as a nondestructive method of determining the lowest detectable  $\beta$ -carotene content in experimentally prepared evaporitic matrices – namely, gypsum, halite and epsomite. Here, 514,5 nm excitation was adopted because of the Resonance Raman enhancement in the carotenoid analysis, and a 785 nm laser source as a more universal wavelength which is now much used in the detection of biomolecules terrestrially. Powdered mixtures were measured directly as well as with a laser beam penetrating the crystals of gypsum and epsomite. The number of registered  $\beta$ -carotene Raman bands differed depending on the particular mineral matrix and the excitation wavelength. Concentrations of  $\beta$ -carotene of about one order of magnitude higher were identified when analyzed through single crystals of gypsum and epsomite, compared to the analysis of the mixtures alone.

**Method:** Synthetic  $\beta$ -carotene was mixed with commercially obtained crystalline powders of three mineral matrices: epsomite ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) and halite ( $\text{NaCl}$ ). The mixtures were pulverized and homogenized in an agate mortar. Powders of five different concentrations of  $\beta$ -carotene were prepared —  $10^4 \text{ mg kg}^{-1}$ ,  $100 \text{ mg kg}^{-1}$ ,  $10 \text{ mg kg}^{-1}$ ,  $1 \text{ mg kg}^{-1}$  and  $0,1 \text{ mg kg}^{-1}$ . The flattened surface of the powdered samples was analyzed soon after preparation to avoid  $\beta$ -carotene decomposition. Laser power was tested to find the optimal conditions – between 15 and 150 mW using the 785 nm excitation source (diode laser) and 0,1 to 10 mW using the 514,5 nm Ar laser.

The following step consisted in analyzing mixtures of  $\beta$ -carotene/evaporite with the laser beam penetrating through the crystals of natural gypsum and synthetic

epsomite (~2 mm thick) in order to evaluate the possibilities of identifying  $\beta$ -carotene within the mineral matrix grains.

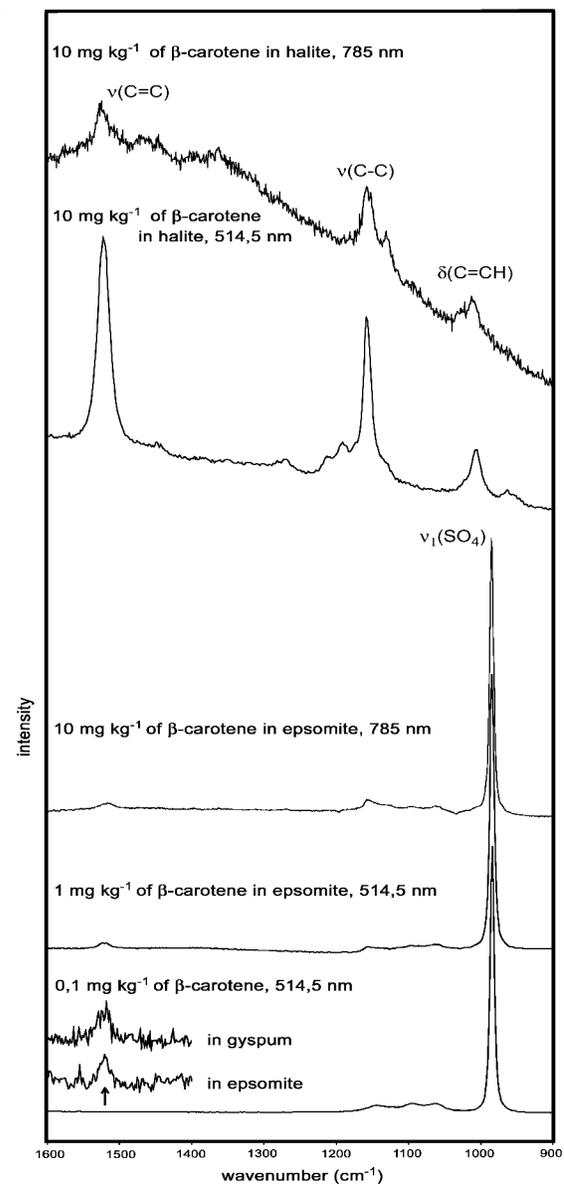


Figure 1: Raman signal of various  $\beta$ -carotene content in the mixtures with halite and sulfate minerals - epsomite and gypsum.

**Results:** At least two  $\beta$ -carotene bands ( $\nu(\text{C}=\text{C})$  around  $1518\text{ cm}^{-1}$  and  $\nu(\text{C}-\text{C})$  at  $1157\text{ cm}^{-1}$ ) were identified using both excitation sources at the  $10\text{ mg kg}^{-1}$  content of  $\beta$ -carotene when analyzed in mixtures with gypsum and epsomite. Measurements using the  $514,5\text{ nm}$  excitation allowed us to detect two  $\beta$ -carotene bands even at the  $1\text{ mg kg}^{-1}$  level in these two matrices. Even at the  $0,1\text{ mg kg}^{-1}$  concentration of  $\beta$ -carotene in sulfate matrix, the  $\nu(\text{C}=\text{C})$  band was observed in the spectra as shown in the figure 1. In the case of the  $\beta$ -carotene/halite mixture, all three characteristic bands of  $\beta$ -carotene were detected at the  $1\text{ mg kg}^{-1}$  concentration level by both excitation sources due to the absence of any Raman signal from the halite, but no feature was observed at the  $0,1\text{ mg kg}^{-1}$  concentration.

Analyses of samples through the monocrystal of gypsum or epsomite, respectively, resulted in the limiting concentration of  $10\text{ mg kg}^{-1}$  necessary for identification of at least some of spectral signatures of  $\beta$ -carotene using the  $785\text{ nm}$  excitation source. However, using  $514,5\text{ nm}$  excitation, two  $\beta$ -carotene features were identified at the  $1155$  and  $1518\text{ cm}^{-1}$  positions in an epsomite matrix under an epsomite crystal even at the  $1\text{ mg kg}^{-1}$  content. Only the  $\nu(\text{C}=\text{C})$  band was identified in the  $\beta$ -carotene/gypsum matrix at the same concentration (figure 2).

**Conclusions:** The analytical potential of Raman microspectrometry has been demonstrated for the identification of  $\beta$ -carotene in a two-component system comprising a biomarker in an evaporitic matrix. Surprisingly low concentrations of  $\beta$ -carotene were identified spectroscopically and nondestructively in the evaporitic mixtures, e.g.  $0,1$ - $1\text{ mg kg}^{-1}$  using  $514,5\text{ nm}$  excitation and  $1$ - $10\text{ mg kg}^{-1}$  using the  $785\text{ nm}$  laser source.

**References:** [1] Squyres S. W. et al. (2004) *Science*, 306, 1709–1714. [2] Squyres S. W. and Knoll A. H. (2005) *Earth Planet. Sc. Lett.*, 240, 1-10. [4] Rothschild L. (1990) *Icarus*, 88, 246-260. [3] Wang A. et al. (2006) *J. Geophys. Res.-Planet*, 111, no. E02S17. [5] Mancinelli R. L. et al. (2004) *Adv. Space. Rev.*, 33, 1244–1246. [6] Edwards H. G. M. et al. (2005) *Analyst*, 130, 917-923. [7] Marshall C. P. et al. (2007) *Astrobiology*, 7, 631-643.

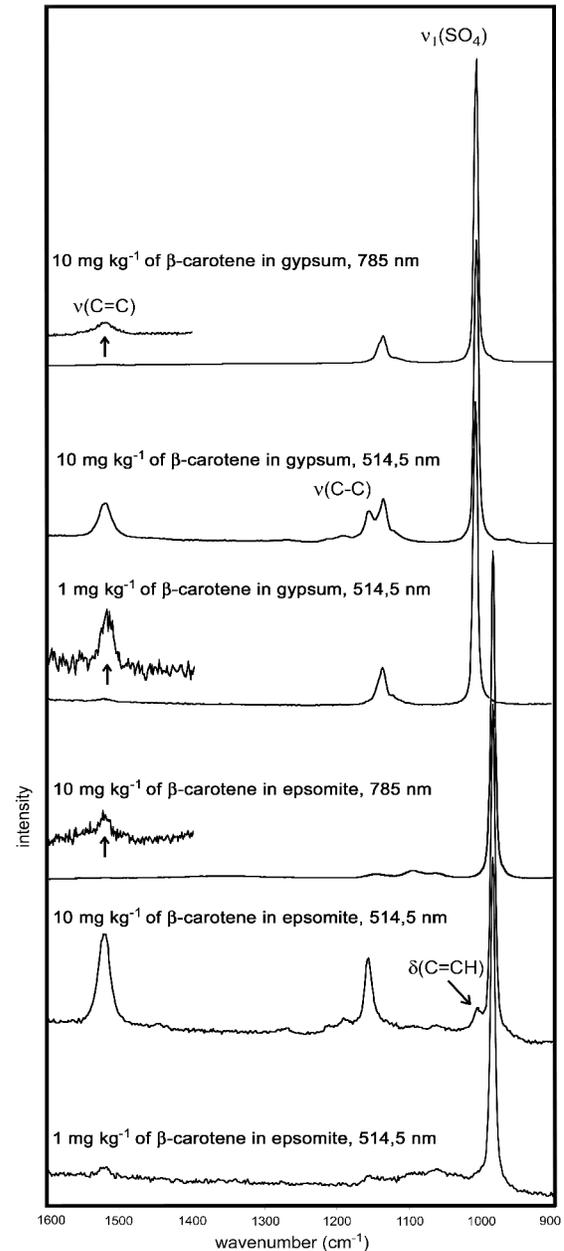


Figure 2: Raman signal of  $\beta$ -carotene as obtained by the analysis of the mixtures with epsomite and gypsum through the monocrystal of these minerals.